RELATIONS BETWEEN THE HEMATOPOIETIC MICROENVIRONMENT, OSTEOGENESIS, AND HEMATOPOIESIS UNDER THE INFLUENCE OF ESTRONE

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The use of estrone in order to produce myelofibrosis in experimental animals has provided a model of the basic phenomena of human myelofibrosis, namely fibrosis of the bone marrow, extramedullary myelopoiesis, an increase in the number of hematopoietic cells (precursors of blood cells), and the potential reversibility of the process [1, 10]. It can accordingly be concluded that the model is adequate for the experimental study of myelofibrosis associated with diseases of the blood system in man.

It was shown previously, during the use of heterotopic bone marrow transplantation, that reduction of hematopoiesis developing parallel with the increase in volume of the bone tissue is unconnected with mechanical displacement of hematopoietic cells [1]. The aim of this investigation was to continue the analysis of the possible mechanisms of the disturbance of hematopoiesis during the development of experimental myelofibrosis.

EXPERIMENTAL METHOD

The investigation was conducted on (C57BL x CBA)F1 mice. Altogether 140 mice were used in three series of experiments. Heterotopic bone marrow transplantation was carried out by the method developed in the laboratories of Professor I. L. Chertkov [4] and A. Ya. Fridenshtein [3]. Estrone was dissolved in olive oil and injected into the experimental animals in a dose of 0.5 mg/kg once a week for 4-6 weeks. Control animals received injections of olive oil alone. The cell content and mass of the femoral bone tissue and of the heterotopic focus were estimated 4-6 weeks after the beginning of the estrone injections and also 60 days after the last injection. In a separate series of experiments a single injection of estrone was given into the donors of the bone marrow, after which their femoral marrow was transplanted. Transplantation of bone marrow was impossible after a large number of injections, due to the development of fibrosis of the bone marrow.

The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

Just as in the original work, when the method of estrogenic myelofibrosis was suggested [10], clear dependence of the reduction of the cell content of the bone marrow on the number of estrone injections (4-6) was revealed (Fig. 1). This indicates that the model of myelofibrosis thus produced is adequate. The same rule as a whole also was observed in a heterotopic focus of hematopoiesis (Fig. 2). Compared with the femoral bone marrow, its cell content after five or six injections of estrone did not differ significantly, probably due to the extremely low values of the cell content of the bone marrow of the heterotopic focus after five injections of estrone. Incidentally, in the control animals, with an increase in the duration of the experiment from 4 to 6 weeks, the cell content of the heterotopic focus increased regularly. Parallel with the reduction of the cell content of the femoral marrow and of the heterotopic focus of hematopoiesis, an increase in the mass of the femur and of the bony capsule of the heterotopic focus was observed in the experimental animals. In the heterotopic focus, unlike in the femur, significant differences in the mass
of bone tissue were discovered only after five injections of estrone. These results raise the question of the causes of suppression of hematopoiesis in the medullary organs and the increase in mass of the bone tissue. Since estrone has no inhibitory action on hematopoietic stem cells [10], the results can be explained either by stimulation of development of osteosclerosis and fibrosis of the bone marrow and the reciprocal relations of these processes and hematopoiesis, or by a disturbance of the stromal microenvironment.

Analysis of the development of bone marrow fibrosis suggests that reciprocal relations exist between the bone tissue and hematopoiesis. However, this was not fully confirmed by the next series of experiments. The mice were not killed immediately after four or six injections of estrone, but not until 60 days had elapsed. The mass of the femur 60 days after the fourth injection of estrone did not differ from that in mice killed immediately after four injections of estrone, and mice of the corresponding control group (Fig. 3). Conversely, the mass of the bony capsule of the heterotopic focus in this situation continued to rise and it differed significantly from that in mice killed immediately after four injections of estrone. It must be recalled that there was also a parallel increase in the mass of the bony capsule in animals of the control group. No significant differences could be found between them (Fig. 4). A definite trend of the cell content of the femoral marrow and heterotopic focus could be distinguished. The number of nucleated cells in the femur increased, but did not reach the level in the control group (Fig. 3). A similar situation also was observed in the heterotopic focus (Fig. 4).

Changes in the cell content of the bone marrow and the weight of the bone when investigated 60 days after six injections of estrone were rather different. For instance, the mass of the femur became slightly less although, however, significantly greater than in animals of the control group (Fig. 3). The mass of the bony capsule of the heterotopic focus declined significantly and did not differ from that in mice of the control group. The character of the changes in the cell content of the medullary organs also